

AMENDMENTS TO THE SPECIFICATION

Please replace the paragraph bridging pages 15 and 16 of the specification with the following corrected paragraph:

Mutagenic plasmid pREG104 was constructed for unmarked gene deletion of *kstR*, the gene encoding a transcription regulator of the *kstD* gene (encoding 3-ketosteroid Δ^1 -dehydrogenase KSTD1) in *Rhodococcus erythropolis* SQ1 (Fig. 1). Briefly, pSDH205 (Van der Geize, R. *et al.* 2000. Appl. Environ. Microbiol. 66:2029-2036) was digested with restriction enzymes *Nru*I and *Bal*I, followed by self-ligation, resulting in plasmid pREG103. An *Eco*RI DNA fragment of pREG103, containing the *kstR* gene deletion was subsequently cloned into *Eco*RI digested pK18mobsacB vector, resulting in pREG104. Unmarked *kstR* gene deletion mutant *R. erythropolis* RG10 was isolated from *R. erythropolis* SQ1 using pREG104 via the sacB counter-selection method as described (Van der Geize R. *et al.* 2001. FEMS Microbiol. Lett. 205:197-202). Genuine *kstR* gene deletion was confirmed by the polymerase chain reaction (PCR) using forward primer (REG-FOR) 5'GGCGACGTTGCCGAGAATT 3' ([SEQ ID NO:4](#)) and reverse primer (REG-REV) 5'TCAGTGTCTGTGAGAGATTCA 3' ([SEQ ID NO:7](#)). A PCR amplicon of 618 bp was obtained with parent strain SQ1 genomic DNA (control). With genomic DNA of *kstR* gene deletion mutant strain RG10 the amplicon was reduced to 393 bp, confirming *kstR* gene deletion.

Please replace the paragraph bridging pages 17 and 18 of the specification with the following corrected paragraph:

A *Rhodococcus* expression vector was constructed for the expression of genes under control of the *kstD* promoter of *R. erythropolis* SQ1 (Van der Geize, R. *et al.* 2000. Appl. Environ. Microbiol. 66:2029-2036). Using the *kstD* promoter, expression of genes in *R. erythropolis* mutant strains harboring a *kstR* gene deletion will be constitutive due to the absence of the repressor of *kstD* expression. The *kstD* promoter region (158 bp) was isolated from *R. erythropolis* SQ1 chromosomal DNA by PCR amplification (25 cycles: 30s 95°C, 30s 64°C, 30s 72°C, using *Taq* polymerase) using forward primer

5' ATAAAGCTTATCGATTATGTGTCCCGCCGCGAAC3' (SEQ ID NO:8) and reverse primer 5' ATAGGTACCATATGTGCGTCCTTACTCCAAGAGGG 3' (SEQ ID NO:9). A *NdeI* site (underlined) was incorporated in the amplicon to be able to clone genes of interest precisely at the ATG startcodon of the *kstD* gene. The amplicon (175 bp) was blunt-ligated into the unique *SnaBI* restriction site of shuttle vector pRESQ (Van der Geize, R. *et al.* 2002. Mol. Microbiol. 45:1007-1018) and the resulting *Rhodococcus* expression vector was designated pRESX (Fig. 2).

Please replace the first full paragraph on page 18 of the specification, starting at line 5, with the corrected paragraph as follows:

The *kstD2* gene, encoding the KSTD2 isoenzyme in *R. erythropolis* SQ1, was isolated from chromosomal DNA of parent strain SQ1 by PCR (conditions: see above), using forward primer 5' GCGCATATGGCTAAGAATCAGGCACCC 3' (SEQ ID NO:10) (*NdeI* site underlined) and reverse primer 5' GCGGGATCCCTACTTCTCTGCTGCGTGATG 3' (SEQ ID NO:11) (*BamHI* site underlined). The introduced *NdeI* and *BamHI* sites were used to ligate the *kstD2* amplicon into *NdeI/BglII* digested pRESX vector. The resulting plasmid was designated pRESX-KSTD2.

Please replace the third full paragraph on page 19 of the specification, starting at line 17, with the corrected paragraph as follows:

The *kshA2* gene was placed under control of the *kstD* promoter in pRESX. In order to achieve this, the *kshA2* gene was amplified from *R. erythropolis* chromosomal DNA as template by PCR using forward primer 5'GGCCATATGTTGACCACAGACGTGACGACC 3' (SEQ ID NO:12) (*NdeI* site underlined) and reverse primer 5'GCCACTAGTTCACTGCGCTGCTCCTGCACG 3' (SEQ ID NO:13) (*SpeI* site underlined). The obtained *kshA2* amplicon was first ligated into *EcoRV* digested pBlueScript (II)KS (pKSH311) and subsequently subcloned as a *NdeI* / *SpeI* fragment into *NdeI* / *SpeI* digested pRESX, resulting in pKSH312.